Efficacy of Avermectins for Root-Knot Control in Tobacco

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ABSTRACT

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The efficacy of three novel experimental compounds—avermectin B_{1a} , avermectin B_{2a} , and avermectin B_{2a} 23-ketone—for control of *Meloidogyne incognita* on tobacco was studied in field plots for two seasons. Application rates ranging from 0.05 to 0.50 kg a.i./ha suppressed root galling and egg production. The number of *M. incognita* eggs per plant was inhibited 21-86% depending on rate and compound. Levels of control achieved by the avermectins were comparable to those of the registered compounds ethoprop and fenamiphos at 6.73 kg a.i./ha.

The widespread use of nematicides to control plant-parasitic nematodes began about 1950 (9). In recent years, environmental and economic considerations have led to a search for more effective, inexpensive, and safe compounds. Although a rigid standardization of methods used to evaluate new nematicides is not desirable, a set of suggested guidelines and procedures for evaluation of these compounds has been developed (6).

The avermectins, a new class of macrocyclic lactones isolated from the soil organism Streptomyces avermitilis (1,7), were originally discovered in the Merck Sharp & Dohme Research Laboratories as anthelmintic agents. They demonstrated high potencies when administered to sheep, cattle, dogs, and poultry infected with a spectrum of gastrointestinal parasites (2,3). Initial indications of insecticidal activity of this group of natural products were shown in tests against the confused flour beetle (Tribolium confusum Duval) (8) and the ectoparasitic larvae of the sheep blowfly (Lucilia cuprina (Wiedemann)) (5). Of the major components of the avermectin fermentation complex, avermectins B1a and B_{2a} are the more effective pesticides. Metabolism studies have shown that avermectin B2a is converted through soil microbial action to the nematicidally active metabolite avermectin B2a 23ketone (V. P. Gullo et al, unpublished).

Preliminary tests indicate that these compounds may be effective in field control of nematodes on a number of

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0191-2917/82/08069103/\$03.00/0 ©1982 American Phytopathological Society agronomic crops. The objective of this study was to evaluate the efficacy of three avermectins on *Meloidogyne incognita* (Kofoid & White) Chitwood on tobacco.

MATERIALS AND METHODS

Small-plot field evaluations of the efficacy of granular formulations of avermectin B_{1a} , avermectin B_{2a} , and avermectin B2a 23-ketone for M. incognita control on tobacco as compared with that provided by ethoprop and fenamiphos were conducted in 1979 and 1980. A portion of a recently cleared field in Wake County, NC, was used for the 1979 test, and part of a nearby field that had previously been in vegetable production was selected as the test site in 1980. Inoculum preparation, field inoculation, design, and tobacco culture were similar both years unless otherwise specified.

A population of *M. incognita* race I was used both years. The nematodes were increased on tomato (*Lycopersicon esculentum Mill.* 'Rutgers') in the greenhouse for 60 days prior to field inoculation. In the preparation of inoculum, the heavily galled tomato root systems were separated from the potting mixture of sandy loam soil and river sand (1:1, v/v) and chopped into approximately

1-cm segments. The segments and potting medium were then thoroughly mixed, and three 500-cm³ composite samples were randomly drawn from the root-soil mixture for an assay of number of eggs and larvae per unit volume.

The experiments were designed as randomized complete blocks with four replicates. Each plot was one row 3.04 m long, and row spacing was 1.2 m. Alleyways 3.04 m wide separated replicates. Lime and fertilizer were added according to soil test recommendations for tobacco.

Each plot was rototilled to a depth of 15 cm prior to inoculation with the nematode. To allow for exact placement of the inoculum, a wooden frame with inside measurements of 0.305 m wide × 3.04 m long was centered over the row to be inoculated, and inoculum was uniformly applied within the frame. The inoculum was incorporated by rototilling each plot twice. Sufficient inoculum was added to each plot to give approximately 100,000 eggs and larvae per 30.5 cm of row.

Two days after inoculation, the wooden frame was again used to ensure exact placement of the test materials in each plot. An improvised applicator (9) was used to apply the chemicals that were incorporated with the rototiller immediately after application. Control plots were inoculated as described earlier, but received no nematicide. Six tobacco (Nicotiana tabacum L. breeding line 1071) seedlings were transplanted in each row immediately following treatment. After 71 days in 1979 and 81 days in 1980, the tobacco stalks were cut at the soil line and the combined fresh weights of stalks and leaves were recorded for each plot. Root systems were dug and rated for galling according to the following scale: 0

Table 1. To bacco yield and root galling in plots treated with avermectin B_{2a} and ethoprop at various rates in 1979

Chemical and formulation		Rate (kg a.i./ha)	Fresh top wt (kg)	Root-gall rating ^y
Avermectin B _{2a}	1G	0.055	4.76 ab ^z	2.37 b
Avermectin B _{2a}	1G	0.168	5.56 b	0.54 d
Avermectin B _{2a}	1G	0.504	4.99 ab	1.08 cd
Avermectin B _{2a}	1G	1.52	4.43 ab	0.13 d
Ethoprop	10G	4.50	4.65 ab	2.83 b
Ethoprop	10G	9.00	3.97 ab	2.04 bc
Control		0	3.63 a	4.54 a

y₀ = no infection, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe.

^z Means followed by the same letter do not differ significantly at P = 0.05 by Waller-Duncan K-ratio t test.

Table 2. Meloidogyne incognita reproduction and tobacco response in inoculated plots after various chemical soil treatments in 1980

Chemical and formulation		v	Rate (kg a.i./ha)	Fresh top wt (kg)	Root-gall rating x	Eggs/root (× 10 ³)	Egg suppres- sions (%) ^y
Avermectin B _{2a}	55 - P990						
23-ketone	0.3G		0.055	$9.42 a^{2}$	2.71 bc	330 cd	61
Avermectin B _{2a}	0.3G		0.055	9.54 a	2.54 bcd	471 bc	44
Avermectin B _{1a}	0.3G		0.168	8.17 a	2.96 b	659 ab	21
Avermectin B _{2a}							
23-ketone	0.3G		0.168	9.87 a	2.63 bcd	273 cd	67
Avermectin B _{2a}	1G		0.168	9.64 a	2.54 bcd	186 cd	78
Avermectin B _{2a}	0.3G		0.168	8.74 a	1.29 de	121 d	86
Avermectin B _{la}	0.3G		0.504	8.28 a	1.54 cde	206 cd	75
Avermectin B _{2a}							
23-ketone	0.3G		0.504	9.31 a	1.29 de	138 d	84
Avermectin B _{2a}	0.3G		0.504	9.19 a	1.04 e	117 d	86
Ethoprop	10G		6.73	9.64 a	1.67 bcde	135 d	84
Fenamiphos	15G		6.73	9.31 a	1.38 cde	58 d	93
Control			0	8.97 a	4.50 a	839 a	

 $^{^{}x}0 = \text{no infection}$, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe.

²Means followed by the same letter do not differ at P = 0.05 by Waller-Duncan K-ratio t test.

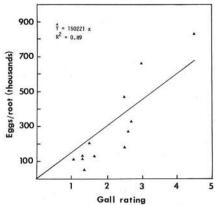


Fig. 1. Relationship between gall rating and number of eggs per plant of *Meloidogyne incognita* on tobacco. 0 = no infection, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = very severe.

= no infection, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe. The amount of reproduction was determined in the 1980 test by extracting the eggs from the roots in each treatment using the sodium hypochlorite method (4).

RESULTS

No differences in tobacco top growth were found between chemical treatments in 1979 (Table 1). Plots receiving avermectin B2a at 0.168 kg/ha yielded significantly more fresh weight than the inoculated control. Root-gall ratings were lower in all treated plots than in the untreated control plots. The lowest average gall rating was in plots treated with avermectin B_{2a} (1G) at 1.52 kg/ha. Treatments of avermectin B2a at rates of 0.168 kg/ha or greater resulted in rootgall ratings significantly lower than the untreated control plot but did not differ from each other. Plots receiving ethoprop at 4.50 and 9.0 kg/ha and avermectin B2a at 0.055 kg/ha had the highest average root-gall ratings of the treated plots. In general, a dose-related suppression of the root-gall development was observed with avermectin B_{2a} application.

In 1980, tobacco top growth was not significantly different for any treatment (Table 2). All chemical treatments except avermectin B_{1a} at 0.168 kg/ha significantly suppressed the production of eggs, and all treatments inhibited root-galling compared with the untreated control. The high rates (0.504 kg/ha) of all three experimental compounds resulted in 75% or greater suppression of egg production. Avermectin B2a (0.3G) at both 0.168 and 0.504 kg/ha and avermectin B_{2a} 23ketone (0.3G) at 0.504 kg/ha resulted in greater than 80% egg suppression and were comparable to the registered compounds ethoprop and fenamiphos at 6.73 kg a.i./ha. Root-gall ratings and egg numbers per root were highly correlated $(R^2 = 0.89)$ (Fig. 1).

DISCUSSION

All three of the macrocyclic lactones—avermectin B_{1a}, B_{2a}, and B_{2a} 23-ketone—were effective in inhibiting root-gall development and reproduction of *M. incognita* on tobacco. Rates ranging from 0.168 to 1.52 kg/ha gave control comparable to that provided by the registered compounds ethoprop and fenamiphos applied at recommended field rates.

Root-gall ratings used in estimating infection levels were highly correlated with actual levels of nematode reproduction. The regression of average numbers of eggs per plant on average gall rating with actual rather than transformed data indicates a trend toward an underestimation of egg numbers at lower gall ratings and an overestimation at the higher end of the scale. A gall-rating scale with more than five categories might decrease the point scatter around the regression line.

The use of root galling as an estimate of nematode reproduction has certain advantages. The expenditure of time and equipment required for gall ratings, especially in field plots, is considerably less than for extraction and counting of eggs. In crops such as tobacco where a high proportion of the root-knot females initiating galls also produce egg masses, the use of root galls as an indication of infection levels is adequate. For less favorable hosts where the proportion of galls to egg masses may be unusually high or low, use of root galls alone may not be adequate.

The small inoculated plot technique for nematicide evaluation has several advantages over larger field tests. Small plots can be established and maintained with a minimum of equipment and land. Because the plots are small, high nematode pressure can be localized and maintained in all plots throughout the study. In farmer fields, where the investigator depends on natural infestations, nematicide evaluation tests usually require much larger plots and six to 10 replicates to minimize variation in distribution and population density of the test nematode. Such tests are expensive and usually depend on farmer's care, which may not be optimum.

These tests appear useful for preliminary evaluation of new experimental compounds. Additional large-scale field tests are necessary for further appraisal of compounds that show a high level of efficacy against a particular nematode species. Such tests would detect differences in efficacy resulting from nematode infestation levels, soil texture, and other variables.

LITERATURE CITED

- Burg, R. W., Miller, B. M., Baker, E. E., Birnbaum, J., Currie, S. A., Hartman, R., Kong, Y. L., Monaghan, R. L., Olson, G., Putter, I., Tunac, J. B., Wallick, H., Stapley, E. O., Oiwa, R., and Omura, S. 1979. Avermectins, a new family of potent anthelmintic agents: Producing organisms and fermentation. Antimicrob. Agents Chemother. 15:361-367.
- Benz, G. W., and Ernst, J. V. 1979. Anthelmintic activities of B_{1s} fraction of avermectin against gastrointestinal nematodes. Am. J. Vet. Res. 40:1187-1188.
- Egerton, J. R., Ostlind, D. A., Blair, L. S., Eary, C. H., Suhayda, D., Cifelli, S., Riek, R. F., and Campbell, W. C. 1979. Avermectins, new family of potent anthelmintic agents: Efficacy of the B_{Ia} component. Antimicrob. Agents Chemother. 15:372-378.
- Hussey, R. S., and Barker, K. R., 1973. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique. Plant Dis. Rep. 57:1025-1028.
- James, P. S., Picton, J., and Riek, R. F. 1980. Insecticidal activity of the avermectins. Vet. Rec. 106:50
- Johnson, A. W. 1978. Test materials and environmental conditions for field evaluation of nematode control agents. Pages 106-108 in: Methods for Evaluating Plant Fungicides, Nematicides, and Bactericides. Am. Phytopathol. Soc.. St. Paul. MN. 141 pp.
- Miller, T. W., Chaiet, L., Cole, D. J., Cole, L. J., Flor, J. E., Goegelman, R. T., Gullo, V. P., Joshua, H., Kempf, A. J., Krellwitz, W. R., Monaghan, R. L., Ormond, R. E., Wilson, K. E.,

y Percentage of reduction in egg numbers compared with the untreated control.

Albers-Schonberg, G., and Putter, I. 1979. Avermectins, a new family of potent anthel-mintic agents: Isolation and chromatographic properties. Antimicrob. Agents Chemother.

15:368-371.

- 8. Ostlind, D. A., Cifelli, S., and Lang, R. 1979. Insecticidal activity of the anti-parasitic avermectins. Vet. Rec. 105:168.
- Taylor, A. L., and Sasser, J. N. 1978. Experimental and agronomic use of nematicides. Coop. Publ. Dep. Plant Pathol., N.C. State Univ. and U.S. Agency Int. Dev. 20 pp.